

USE OILS HAVING A HIGH LAURIC ACID CONTENT AS AN ANIMAL FEED**BACKGROUND OF THE INVENTION**

The invention relates to the field of livestock production. About one quarter of antibiotics dispensed in the United States are administered in sub-therapeutic doses to promote weight gain in apparently healthy livestock. Increasingly, the use of antibiotics at sub-therapeutic levels in livestock has been implicated in the development of antibiotic resistance in bacteria, including human pathogenic bacteria and foodborne bacteria. J. Raloff, 1998, *Science News* 154:39; J. Raloff, 1999, *Science News* 155:356. Additionally, antibiotic residues in meat products is also of concern. Therefore, effective methods and compositions for reducing the use of antibiotics in the agricultural sector are needed.

SUMMARY OF THE INVENTION

In accordance with the current invention, methods and compositions for reducing or eliminating the use of antibiotics to promote the growth of animals have been discovered. The methods and compositions relate to the use of an anti-microbial fatty acid component as part of the animal feed, either combined with the feed or administered separately as a feed supplement. The methods and compositions of the invention allow for the elimination or reduction of the use of antibiotics as growth promoters in animals. This reduction or elimination is important for several reasons, including but not limited to the following: (1) use of antibiotics as growth promoters in animals is known to contribute to the development of antibiotic resistance in microorganisms, particularly foodborne microorganisms; (2) use of antibiotics as growth promoters in animals results in antibiotic residue in animal tissue used for human food; and (3) use of antibiotics as growth promoters contributes to the introduction into the ecosystem of mutant, antibiotic resistant forms of common organisms, through the exposure of microorganisms to antibiotics in the animal and to antibiotics excreted in fecal matter. Therefore, in allowing for the elimination or reduction in the use of antibiotics in livestock management and in the management of

other production animals, the methods and compositions of the invention address a pressing problem in the agricultural sector.

One aspect of invention is a method for reducing the antibiotic content of animal feed comprising feeding the animals an anti-microbial fatty acid component as part of the animal feed, either combined with the feed or administered separately as a feed supplement. Animal feed, as used herein, includes all solid or semi-solid feeds, as well as liquid feed such as milk-replacers. The anti-microbial fatty acid component may comprise at least one fatty acid or glyceryl ester of a fatty acid, or a derivative thereof, which has an anti-microbial activity and which is suitable for use as animal feed. The anti-microbial fatty acid component may also comprise at least one precursor to a fatty acid or glyceryl ester of a fatty acid, or derivative thereof, having anti-microbial activity, provided the precursor is suitable for use as animal feed and is convertible to a form that is active as an anti-microbial by the animal's digestive process. Preferred anti-microbial fatty acid components, either provided directly to the animal or in precursor form, are those described in Kabara et al. (U.S. Pat. No. 4,002,775), Iwasaki et al. (4,961,934), Olund et al. (U.S. Patent No. 5,550,145), and Hayashi (U.S. Patent No. 5,462,967). For example, preferred anti-microbial fatty acid components, either provided directly to the animal or in precursor form, are lauric acid, monolaurin, other medium chain length fatty acids (C6 to C14) and glyceryl monoesters thereof, and palmitoleic (C16:1) and oleic acid (C18:1), and glyceryl monoesters thereof. Preferred precursor forms of fatty acids or glyceryl monoesters of fatty acids are diglycerides and triglycerides having lauroyl substituents.

Anti-microbial, as used herein, refers to a compound or composition having measurable activity against any of bacteria, fungi, viruses, protozoa, etc., or combinations thereof. Assays for describing such activity of fatty acid components are well known in the art and are described for example in Fletcher et al., 1985, Effect of monoglycerides on *Mycoplasma pneumonia* growth, in *The Pharmacological Effects of Lipids II* (J.J. Kabara ed., The American Oil Chemists' Society, Champaign, Illinois); Schemmel and Kabara, 1985, Fatty Acids, Monoglycerides and Sucrose Esters as Anticaries Agents Review, in *The Pharmacological Effects of Lipids II* (J.J. Kabara ed., The American Oil Chemists' Society, Champaign, Illinois);

Kabara, 1985, Inhibition of *Staphylococcus aureus* in a model sausage system by monoglycerides, in *The Pharmacological Effects of Lipids II* (J.J. Kabara ed., The American Oil Chemists' Society, Champaign, Illinois); Sands et al., 1978, Antiviral effects of fatty acids and derivatives; lipid-containing bacteriophages as a model system, in *The Pharmacological Effects of Lipids I* (J.J. Kabara ed., The American Oil Chemists' Society, Champaign, Illinois); Li and Kabara, 1978, Effects of lauricidin on *Fomes annosus* and *Phellinus weirii*, in *The Pharmacological Effects of Lipids I* (J.J. Kabara ed., The American Oil Chemists' Society, Champaign, Illinois); Kabara, 1978, Fatty acids and derivatives as antibiotic agents; A review, in *The Pharmacological Effects of Lipids I* (J.J. Kabara ed., The American Oil Chemists' Society, Champaign, Illinois);

A preferred anti-microbial fatty acid component contains lauric acid or monolaurin or contains precursors for lauric acid or monolaurin. A particularly preferred anti-microbial fatty acid component comprises natural oils wherein a high percentage of fatty acid substituents on the triglycerides, diglycerides and monoglycerides are lauroyl substituents (referred to herein as "high lauric acid oils"). High lauric acid oils preferably comprise at least about 20% lauric acid as acylated fatty acids in the triglyceride, more preferably at least about 40%, and most preferably at least about 50%. Such natural oils include, but are not limited to coconut oil, rapeseed oil, palm kernel oil, murumuru tallow, and tucum oil. As used herein natural oils means any oil derived from plant or animal material including, but not limited to, oils that are derived from plants that have been genetically modified either through traditional breeding or through genetic engineering techniques. As one example, canola oil, rapeseed oil or soybean oil, derived from plants that have been genetically modified to have a high lauric acid content may be employed. Such an oil is available, for example, under the tradename Laurical® from Calgene Inc., Davis, California. When high lauric acid oils are ingested by an animal, the digestive enzymes release lauric acid and monolaurin. The lauric acid and monolaurin are then either absorbed through the intestinal wall into the blood stream or remain in the digestive tract. Therefore, the lauric acid or monolaurin can exert an anti-microbial effect systemically within the animal or within the digestive tract.

In one aspect of the invention, high lauric acid oil will be employed as part of the feed or as a feed supplement such that lauric acid will preferably comprise about 0.5% to about 10% of the animal feed, more preferably about 2% to about 7%, and most preferably about 3% to about 5% of the feed. For high lauric acid oils which contain approximately 50% lauric acid as acylated fatty acids, such as coconut oil or palm kernel oil, the feed will typically comprise about 1% to about 20% high lauric acid oil, preferably about 4% to about 14%, and more preferably about 6% to about 10%.

Animals, as used herein, includes, but is not limited to, farm livestock, pets, and any animals produced for human consumption. Preferred animals include poultry (chickens, turkey, ducks, ostrich, emu, quail etc.), fish, pigs, lambs, veal calves, dairy calves, beef calves, and any other monogastric livestock. Preferred animals are production animals. Production animals, as used herein, are animals which produce food for human consumption. Animals, as used herein, also refers to ruminant animals. However, for ruminant animals, it is desirable that the fatty acid component be rumen protected, so as not to exert deleterious effects on the bacteria in the rumen. Methods of rumen protection are described, for example, in Vinci et al. (U.S. Patent No. 5,182,126), Cummings et al. (U.S. Patent No. 5,250,307), LaJoie et al. (U.S. Patent No. 5,874,102), which are hereby incorporated by reference.

In one aspect of the invention, a high lauric acid oil is added to the feed of poultry, particularly, either as feed or as a feed supplement, to promote the health of the chickens or to prevent bacterial contamination of poultry products, including contamination by *Salmonella typhimurium*. Poultry products include any part of the bird that is used for human consumption, as well as egg products. Preferred poultry includes chickens and turkeys. According the invention, sufficient high lauric acid oil will be included in the diet of the chickens such that lauric acid will preferably comprise about 0.5 % to about 10 % of the diet, more preferably about 2 % to about 7 %, and most preferably about 3 % to about 5 %.

According to the invention, methods and compositions for achieving a high feed efficiency with reduced amounts of antibiotics are described. Feed efficiency, as used herein and as generally known in the art, refers to the following ratio: weight

- 5 -

gain of the animal/weight of food ingested. In one aspect of the invention, preferably at least 85%, and more preferably at least 90%, and most preferably at least 95%, of the optimal feed efficiency achievable with any particular diet and antibiotic supplement (optimal antibiotic supplement), can be achieved with less than 50% the optimal antibiotic supplement by the supplementation of the diet with lauric acid. In another aspect of the invention, preferably at least 85%, more preferably at least 90%, and most preferably at least 95%, of the maximal feed efficiency achievable with any particular diet and the maximum antibiotic supplement allowable under Title 21 of the Code of Federal Regulations, Section 558 (allowable antibiotic supplement), can be achieved with less than 50% the allowable antibiotic supplement by the supplementation of the diet with lauric acid. It is of course recognized that the allowable antibiotic supplement for increasing feed efficiency will depend on the type of animal and the use of the animal. For example, 21 CFR § 558 may specify a different allowable antibiotic supplement for broiling chickens and laying chickens. Title 21 of the Code of Federal Regulations, Section 558, entitled "New Animal Drugs for Use in Animal Feed", is hereby incorporated herein by reference.

It is of course recognized by one of skill in the art that the lauric acid supplement to the diet, typically in the form of a natural oil, may replace other fats in the diet. A high feed efficiency (e.g. $\geq 85\%$) is preferably achieved with less than about 50% of the optimal antibiotic supplement, more preferably with less than about 40% of the optimal antibiotic supplement, and most preferably less than about 30% of the optimal antibiotic supplement. Additionally, a high feed efficiency (e.g. $\geq 85\%$) is preferably achieved with less than about 50% of the allowable antibiotic supplement, more preferably with less than about 40% of the allowable antibiotic supplement, and most preferably less than about 30% of the allowable antibiotic supplement.

In another aspect of the invention, high feed efficiency is preferably achieved with less than about 50% of the usual antibiotic supplement for a particular application, more preferably with less than about 40%, and most preferably with less than about 30%. The usual antibiotic supplement for a particular application can be readily determined by one of skill in the art.

- 6 -

According to the invention, methods and compositions for achieving control of *Salmonella typhimurium* in poultry with reduced amounts of antibiotics are described. Control of *Salmonella typhimurium*, as used herein, is determined by assaying for the presence of *Salmonella typhimurium* in the chickens, using techniques that are well known in the art. As is well known in the art, assays can be conducted, for example, on fecal droppings from the animals or through the culture of intestinal bacteria or bacteria present in the oviduct in the animals. Assays of oviduct bacteria would be important for determining the effects of methods and compositions of the invention for reducing the risk of contamination of the interior of eggs with *Salmonella typhimurium*. In one aspect of the invention at least 85%, preferably at least 90%, and more preferably at least 95%, of the optimal *Salmonella typhimurium* control achievable with any particular diet and antibiotic supplement (optimal antibiotic supplement for control of *Salmonella typhimurium*), can be achieved with less than 50% the optimal antibiotic supplement for control of *Salmonella typhimurium* by the supplementation of the diet with lauric acid. In another aspect of the invention at least 85%, preferably at least 90%, and more preferably at least 95%, of the maximum *Salmonella typhimurium* control achievable with any particular diet and the maximum antibiotic supplement allowable under Title 21 of the Code of Federal Regulations, Section 558 (allowable antibiotic supplement for control of *Salmonella typhimurium*) can be achieved with less than 50% the allowable antibiotic supplement for control of *Salmonella typhimurium* by the supplementation of the diet with lauric acid. It is of course recognized that the allowable antibiotic supplement for controlling *Salmonella typhimurium* will depend on the type of animal and the use of the animal. For example, 21 CFR § 558 may specify a different allowable antibiotic supplement for broiling chickens and laying chickens.

Control of *Salmonella typhimurium* (e.g. $\geq 85\%$) is preferably achieved with less than about 50% of the optimal antibiotic supplement for control of *Salmonella typhimurium*, more preferably with less than about 40%, and most preferably less than about 30%. Additionally, control of *Salmonella typhimurium* (e.g. $\geq 85\%$) is preferably achieved with less than about 50% of the allowable antibiotic supplement

- 7 -

for control of *Salmonella typhimurium*, more preferably with less than about 40%, and most preferably less than about 30%.

5 In another aspect of the invention, control of *Salmonella typhimurium* is preferably achieved with less than about 50% of the usual antibiotic supplement for a particular application, more preferably with less than about 40%, and most preferably with less than about 30%. The usual antibiotic supplement for a particular application can be readily determined by one of skill in the art.

10 Also contemplated as part of this invention are animal feed compositions comprising a lauric acid component and a minimal or reduced antibiotic level as described above.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure.

15

EXAMPLES

In the following examples, all parts and percentages are by weight unless otherwise indicated.

Example 1 - Determination of optimal type and amount of a fatty acid component.

20 For any given animal type the optimal type and amount of fatty acid component for the diet can be determined using knowledge and skills that are well known in the art.

25 However, as a specific example, the optimal protocol for reducing or eliminating antibiotics in chicken feed through the use of coconut oil as a feed supplement would be determined as follows, for a particular breed of chicken. Three groups of chickens are evaluated. All groups are fed normal or experimental starter diets, which are switched to the experimental or normal growth and finishing diets at the proper ages. The starter diets are fed to the chickens for days 0-15 after hatching and consist of about 26% crude protein and 6% fat. The growth diet (days 15-32) is

adjusted to decrease the protein content to about 20% and to increase the fat content to about 7%. A finishing/withdrawal diet is fed to the chicks for days 33-42 and this diet consists of the growth diet with, with exception that antibiotics are eliminated from the diet for those groups receiving antibiotics.

5 A Ross x Ross Hiyield strain of chicken (broiler chickens) are used for the study. The birds are housed with litter for the first 15 to 18 days and then transferred to pens. The experiment involves six birds per group, three assortment groups (one control group and two treatment groups), and eight replicates, for a total of 144 birds. At the end of the starter period (day 15), two birds from each group will be sacrificed
10 for assay purposes.

 The first group, a control group, is fed the normal diet, including the antibiotic supplements which are ordinarily a part of the normal diet. The second group is fed the normal diet, with no antibiotics, and with some of the fat in the diet replaced with coconut oil, so that lauric acid would comprise about 3% by weight of the diet. The
15 coconut oil can be applied to the feed pellets after extrusion in amounts appropriate for the diet formulation. Since coconut oil contains about 50% lauric acid, addition of coconut oil to the diet to comprise about 6% would yield a diet with 3% lauric acid. For Ross x Ross Hiyield chickens, the diet would provide about 3.6 g/day of lauric acid. The third group would receive the lauric acid supplemented diet and one half of
20 the usual amount of antibiotics. For example, typical antibiotics employed for growth promotion in chickens include the following: narasin/nicarbazin (tradename Maxiban/BMD), there is a five day withdrawal period before the birds can be killed for food; chlortetracycline with salinomycin, typically employed in an amount of 0.05 to 0.1 lb/ton of feed, there is one day withdrawal period before the birds can be killed
25 for food; monensin (tradename Coban), typically employed at a rate of 0.1 lb/ton of feed; and Zn Bacitracin, typically employed in an amount of 0.4 lb/ton, there is a five day withdrawal period before the birds can be killed for food.

 The growth parameters of the chicks are monitored twice a week. Monitoring includes measurements of body weight, length, general health, and leg and foot
30 development, etc. At the end of the experiment, when the birds reach market weight, the birds are sacrificed for assay purposes. The chickens sacrificed at day 15 and the

market weight chickens are assayed as follows: the intestinal contents are cultured for *Salmonella*, *E. coli*, and other coliform bacteria; fecal cultures are taken; bone mineralization is determined; intestinal brush border enzymes are analyzed; samples of the jejunum are analyzed by electron microscope examination; and intestinal lymphocyte function is analyzed.

The optimal combination of the fatty acid component with a particular antibiotic, or combination of antibiotics, may also be determined. The fatty acid component is combined with 0%, 20%, 30%, 40,% and 50% of the usual antibiotic supplement and the chickens are evaluated as described above to determine the minimal level of antibiotic which, in combination with the fatty acid component, yields optimal growth of the chickens.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The preceding preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The entire disclosure of all patent applications, patents, and publications cited herein are hereby incorporated by reference.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention and, without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various uses and conditions.